# tRNA family-specific, stress-specific changes in tRNA intron turnover

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**ABSTRACT**

Transfer ribonucleic acids (tRNAs) are abundant molecules, comprising ~15% of cellular RNAs. Although the major biological role for tRNAs is to bring amino acids to the ribosome during protein synthesis, they also play many secondary roles. Defects in pre-tRNA biogenesis and processing cause numerous disorders, from neurodegenerative diseases to cancer. In eukaryotes, a subset of tRNA-encoding genes contain non-coding introns that must be removed in post-transcriptional tRNA processing; in *S. cerevisiae*, these account for 20% of pre-tRNAs. Through an unbiased screen of the yeast genome, my lab identified two proteins required for the turnover of the intron derived from $tRNA^{IleUAU}$, the tRNA employed in the screen (Wu et. al. 2015). Wu and Hopper showed that the free $tRNA^{IleUAU}$ intron is first phosphorylated on the 5' end by the tRNA ligase/kinase Rlg1, then degraded in the 5' to 3' direction by the exonuclease Xrn1. Rlg1 also ligates the mature tRNA halves (Wu and Hopper 2014). For the remaining intron-containing tRNAs, intron turnover is only Rlg1-dependent, only Xrn1-dependent, or neither dependent on Rlg1 nor Xrn1 (Bao and Hopper, unpublished data). The existence of multiple pathways for intron turnover provides evidence that this is an important process. Taken with the conservation of tRNA introns from Archaea to humans, there seems to be an evolutionary selection for the presence of them in cells. If so, levels of tRNA introns may be regulated in response to various environmental conditions as a mechanism to handle cellular stress. I have tested the effects of various environmental stress conditions including oxidative stress, glucose deprivation, and heat shock on tRNA intron levels in yeast, as assessed by Northern blot analysis. My data show that the free $tRNA^{TrpCCA}$ intron accumulates immediately and significantly upon exposure to hydrogen peroxide stress. Likewise, I observe that $tRNA^{ProUGG}$ accumulates immediately upon exposure to 42°C heat shock. Conversely, I report

that the free $tRNA_{IleUAU}$ intron is degraded more rapidly when yeast cells are starved for glucose. My data suggests that the accumulation or accelerated degradation of free tRNA introns is specific to the family of free tRNA intron, as well as specific to the stress condition, suggesting that tRNA introns may play an important role in the cellular response to stress.

## INTRODUCTION

Transfer ribonucleic acids (tRNAs) are adaptor molecules that carry amino acids to the ribosome for protein synthesis. The appropriate amino acids are transported by tRNAs to messenger RNAs (mRNAs) on ribosomes based on the codons in the mRNA. All eukaryotic cells contain tRNA introns and their removal from tRNA molecules via splicing is essential for deciphering the genetic code. Splicing occurs in the nucleus in studied vertebrates, but intriguingly, it occurs on the cytoplasmic surface of the mitochondria in budding and fission yeast (Wan and Hopper 2018). Removal of tRNA introns is catalyzed by the tRNA splicing endonuclease complex. Under optimal growth conditions in wild type cells, this process occurs efficiently and the excised introns are rapidly degraded. For this reason, free tRNA introns have been rarely detected in cells in nature. Many features of tRNA introns and their turnover processes remain unknown and a main purpose of my project is to identify a potential evolutionary advantage for maintenance of tRNA introns.

Aside from their essential role in the translation of messenger RNA (mRNA) to protein, tRNAs also participate in various regulatory roles within cells. These roles range from stress response to cell metabolism to cell proliferation. More recently, studies have shown that the dysregulation of translation can lead to the development of cancer. RNA polymerase III is responsible for the transcription of tRNAs, and its expression is regulated by the oncogenic signaling pathways AKT-mTOR, RAS-MAPK, and MYC (Zhang et al. 2018). Thus, oncogenic signaling pathways can alter tRNA expression in cells. An increased tRNA pool can function to accelerate translation by supplying biomolecules necessary for tumor metabolism. Studies by Zhang et. al. found the overexpression of tRNAs in multiple cancer cell types. Interestingly, similar expression levels and patterns of expression of tRNA families were observed across

different cancer cell types. For example, breast cancer, among 8 other cancer types, showed up-regulation of tRNA expression. tRNA abundance and modification are closely linked to protein expression.

Additionally, tRNAs and tRNA fragments have the ability to bind to cytochrome c, inhibiting its function as an intermediate in apoptosis (Hou and Yang 2013). Cytochrome c activates apoptotic proteins called caspases which degrade cells. This suggests that tRNAs play a central role in suppressing apoptosis. Because tRNA introns are fragments released from tRNAs, they may also have the ability to bind cytochrome c. Other tRNA derivatives have been shown to play a role in proliferation, metastasis, and invasiveness of cancer. In several cancers, there is increased modification of tRNAs by tRNA-modifying enzymes (Zhang et al. 2018). tRNA processing is essential for functioning in the translation of RNA to protein. It has been found that under oxidative stress, tRNAs are cleaved into smaller molecules which have the unique ability to either suppress or enhance translation initiation (Ivanov et al. 2011; Kim et al. 2017). Kim et al. (2017) investigated the inhibition of a particular tRNA-derived small RNA and observed the induction of apoptosis in a patient-derived orthotopic hepatocellular carcinoma model in mice. Oxidative stress also causes deactivation of the 3’ CCA tail, globally shutting down translation (Czech et al. 2013). Because I am also studying tRNA fragments under oxidative stress, I anticipate that tRNA introns have the potential to play similar roles in the cell.

The Hopper lab studies the trafficking and processing of tRNAs in budding yeast, *Saccharomyces cerevisiae*. There are many advantages to studying yeast, including that yeast is a unicellular eukaryotic organism, and the fundamental processes of pre-tRNA splicing and intron turnover are conserved in all eukaryotes. Also, the complete yeast genome has been sequenced and studied since 1996. The study of tRNA processes in yeast has been made possible and

efficient through the creation of mutant collections as well as plasmid collections. Mutant collections allow for the study of particular genes while plasmid collections allow manipulation of the genetic material in the open reading frame of a particular cell. Moreover, a large number of protein-encoding sequences have homologs in the human genome, facilitating the study of human disease.

Previously, the Hopper lab conducted a genome-wide screen of *S. cerevisiae* seeking novel genes that encode proteins involved in tRNA processing (Wu et. al. 2015) (Fig. 1A). $tRNA^{IleUAU}$ was used as the tRNA reporter for this screen because it contains the largest intron (60 nucleotides), allowing for tRNA processing intermediates to be clearly visualized by Northern blot analysis. This screen led to the discovery of two proteins involved in the tRNA intron turnover process and the subsequent delineation of the mechanism for the $tRNA^{IleUAU}$ intron degradation. Following splicing of the intron from the tRNA by the splicing endonuclease complex, the 5' end is phosphorylated by Rlg1, a tRNA ligase and kinase which is also responsible for the ligation of the tRNA exons. The intron is then degraded in the 5' to 3' direction by Xrn1, an exonuclease, resulting in free nucleotides (Fig. 1B,C). The kinase activity of Rlg1 adds a phosphate to the 5' terminus of the freed $tRNA^{IleUAU}$ intron, allowing its degradation by the Xrn1 5' to 3' ribonuclease, which requires a 5' phosphate (Wu and Hopper 2014). Therefore, free $tRNA^{IleUAU}$ introns accumulate in mutant yeast lacking *XRN1* (*xrn1Δ*), and in strains harboring a temperature sensitive allele of the essential *RLG1* gene (*rlg1-4*) (Fig. 1B,C).

Continued study of these two proteins' involvement in the turnover of the remaining 9 intron-containing tRNA genes by a former member of the Hopper lab, Alicia Bao, has led to the discovery of multiple intron turnover pathways. These pathways were delineated based on the

relative accumulation of free tRNA introns in the *xrn1Δ* strain and in the *rlg1*-4 strain. Accumulation of free tRNA introns in these backgrounds means that mutating these enzymes prevents intron turnover from occurring, and thus indicating the roles of these enzymes in the degradation process. In addition to the $tRNA_{IleUAU}$ intron, the $tRNA_{LeuCAA}$ and the $tRNA_{LysUUU}$ introns are also dependent on both Rlg1 and Xrn1, based on the accumulation of these tRNA introns in both mutant backgrounds (Fig 2A,B). However, the degradation of another category of tRNAs is dependent only on Rlg1, as indicated by a more prominent accumulation of these free introns in the *rlg1*-4 strain than in the *xrn1Δ* strain. This observation also suggests that there is at least one unknown nuclease that mediates the degradation of this family of tRNA introns. Conversely, a more prominent accumulation of free introns is seen in the *xrn1Δ* strain than in the *rlg1*-4 strain for yet a third category of tRNAs with intron turnover mechanisms dependent only on Xrn1. This suggests that there is at least one unknown kinase that mediates the degradation of these tRNA introns. The degradation of a fourth category of tRNA families is independent of both Rlg1 and Xrn1, as evidenced by the lack of tRNA intron accumulation in either mutant background (Fig. 2A,B). Thus, seven of the ten families of intron-containing tRNA families have unknown intron turnover mechanisms.

While studying the exonuclease activity of Xrn1 and the kinase activity of Rlg1 in the degradation of the $tRNA_{TrpCCA}$ family via Northern blot analysis, Alicia Bao noticed a band migrating below the $tRNA_{TrpCCA}$ free intron band that also hybridized to the intron probe. She was able to perform experiments which translated to four separate lines of evidence for this unknown band to be a circular form of the $tRNA_{TrpCCA}$ intron (Bao and Hopper, unpublished data). Degradation of this circular tRNA intron would require yet another mechanism for

degradation involving an unknown endonucleolytic RNase. The presence of multiple intron turnover mechanisms in yeast underscores its biological importance.

The presence of tRNA introns in all eukaryotic cells, from Archaea to humans, suggests an unknown evolutionary advantage of them. tRNA introns remain short sequences in all cells. Their location in the anticodon loop of the precursor-tRNA also remains conserved (Chan & Lowe, 2009, 2016). However, just as different organisms contain variation in the number of tRNA genes encoded in the genome, the percentage of these genes that contain introns also differs. For example, while about 20% of tRNA genes in yeast contain introns, only about 7% of tRNA genes in humans contain introns (Chan & Lowe 2009, 2016). Despite the conservation of the presence of tRNA introns over evolution, the sequences of tRNA introns are not well conserved (Chan & Lowe, 2016). Interestingly, Lu et. al. (2015) discovered some sequence similarity between tRNA introns of closely-related species, such as the particularly large $tRNA_{Tyr}$ intron among Drosophilids that diverged 50-60 million years ago. Nonetheless, in all cells, tRNA introns are rapidly and efficiently turned over, yet very little remains known about this process. No one understands why tRNA introns are conserved from Archaea to human cells as they provide no opportunity for alternative splicing and render tRNAs nonfunctional until they are removed. One idea is that tRNA introns might serve a biological function and, if so, there may be some conditions in which they avoid rapid turnover so that they may fulfill such a function.

Naturally, yeast are exposed to endless environment stressors, and my project seeks to mimic these stressors in order to test the hypothesis that tRNA introns may accumulate upon various stress conditions, thereby evading degradation and perhaps providing function for cells. The stress conditions I investigate include ethanol, oxidative, heat, osmotic, glucose starvation,

rapamycin treatment, and saturated growth. These environmental conditions were chosen based on their previously reported roles in the regulation of RNA processing. For example, Thompson et. al. 2008 showed that oxidative stress induces tRNA cleavage, and Morgan et. al. 2019 showed that rapamycin-mediated inhibition of TORC1 leads to the stabilization of linear mRNA introns. My preliminary data show the accumulation of certain free tRNA introns in the budding yeast model system under oxidative and other stresses. My data in combination with other studies from my lab provide support for the model that tRNA introns may serve a novel function in yeast, which would define a new category of regulatory noncoding small RNAs and also provide a reason for the conservation of intron-containing tRNA genes throughout eukaryotes.

## MATERIALS AND METHODS

### *Strains and Media*

Wild type strains in the MATa/BY4741 background were purchased from Open Biosystems. YEPD media (yeast extract, trypticase peptone, dextrose, supplemented with adenine [0.04g/L], uracil [0.04g/L]) was used for all experiments. Ethanol-stress media contained 5% 200 proof EtOH. Oxidative-stress media contained 3mM $H_2O_2$. Cells subjected to temperature stress were shifted to a 37°C incubator, and cells subjected to heat shock were shifted to a 42°C water bath. Salt-stress media contained 0.5M NaCl. To obtain glucose starved cells, log-phase cells were shifted to YEPD media lacking dextrose. Rapamycin-stress media contained 100nM rapamycin. Saturated growth was achieved by growing cells to late-log and early stationary phase (0.8-1.0 $OD_{600}$).

### *RNA isolation*

Yeast strains were grown in YEPD at 23°C to mid-log phase (0.4-0.6 $OD_{600}$). Phenol extraction was used to isolate small RNAs as described in Wu et. al. 2013. Remaining yeast cultures were shifted to media containing environmental stressors and small RNAs were isolated from aliquots every 30 minutes up to 120 minutes. An additional 15mL of yeast culture was grown for 2 hours without various stressors and then RNAs were isolated by phenol extraction (mock).

### *Northern blotting analysis*

A 10% polyacrylamide gel was used to separate 10 μg of small RNAs at 40-60V for 20-22 hours. RNAs were transferred to a Hybond N+ membrane and detected with non-radioactive digoxygenin-labeled probes complementary to the full introns (Wu et. al. 2013) (Table 1). A Lumi-Imager (Boehringer Mannheim) was used to detect the chemiluminescent signal. Quantification of the intensity of the ratio of free intron species vs. 5S rRNA as the loading

controls was achieved using ImageJ. 5S rRNA was chosen as the control for quantification because it is a stable transcript of RNA polymerase III, thereby serving as a loading control even if there are changes in transcription during the time course of stress induction. The tRNA initial transcripts were an additional candidate for control, but because environmental stresses are known to affect the tRNA gene pool, this method would not have been able to indicate isolated changes in tRNA intron turnover. Decreases in tRNA intron levels could have been artifacts of decreases in the transcription of particular tRNAs. Additionally, stress impacts tRNA modification which affects tRNA turnover, which is why mature tRNA was not employed as the internal control. However, several Northern blots show high levels of tRNA introns despite substantial decreases in the transcription of new tRNAs. In these cases, changes in tRNA intron turnover is likely even more exacerbated than the quantification to 5S rRNA indicates. Thus, additional quantification using pre-tRNAs as controls should be conducted in the future. Graphs were created and statistics were performed using GraphPad Prism 8.

## RESULTS

### *Discovery of tRNA intron accumulation under environmental stress*

The lab first incidentally identified a tRNA family-specific difference in tRNA intron levels while studying the enzymes involved in various mechanisms of tRNA intron turnover. Use of the temperature sensitive *rlg1-4* mutant required the evaluation of all 10 intron-containing tRNAs in 23°C and 37°C environments in wild-type, *xrn1Δ,* and *rlg1-4* backgrounds. Interestingly, the levels of $tRNA_{LysUUU}$ intron at 37°C were increased in all three strains compared to the levels of free intron at 23°C (Bao and Hopper, unpublished data) (Fig. 1B). Furthermore, the same phenotype was not observed in other intron-containing tRNAs. Thus, it appears that some free $tRNA_{LysUUU}$ intron accumulate at the elevated temperature of 37°C. This observation led us to expand our investigation to additional stressors and individual tRNA families.

### *Analysis of 37℃ temperature stress*

As previously noted, while categorizing the turnover mechanism of each tRNA family, the $tRNA_{LysUUU}$ intron was found to accumulate under 37°C temperature stress. Repetition of this experiment with a 120 minute time course did not yield statistically significant results. I observe that there is a slightly increased level of free $tRNA_{LysUUU}$ intron after 120 minutes in a 37°C environment compared to the level of intron in the 120 minute mock treatment group grown at 23°C, that is detectable on the Northern blot and graphical quantification (Fig. 3A). Additional repeats of this experiment need be conducted in order to determine if the degradation of free $tRNA_{LysUUU}$ introns is affected by the shift to a 37°C environment. Also, the $tRNA_{LysUUU}$ intron does not appear to accumulate upon immediate exposure to heat; only after 90 minutes is there a visible increase in free $tRNA_{LysUUU}$ intron levels. Contrary to the results of the free $tRNA_{LysUUU}$

intron levels under temperature stress, the free tRNA$^{LeuCAA}$ intron appears to be downregulated in the presence of 37°C temperature stress, while pre-tRNA levels are unchanged (Fig. 3B). Investigation of this tRNA family was conducted because tRNA$^{LeuCAA}$ is in the same category of intron turnover mechanism as tRNA$^{LysUUU}$—both families' turnover mechanisms are dependent on Rlg1 and Xrn1 (Fig. 2B). While the 0 minute and the 120 minute mock treatment groups have visible intron bands, the temperature stressed lanes contain lesser amounts of free intron. Thus, while tRNA$^{LeuCAA}$ introns may be more efficiently degraded upon exposure to 37°C temperature stress, tRNA$^{LysUUU}$ introns may accumulate after some time, or their turnover may be unaffected by 37°C temperature stress.

*Analysis of 42℃ heat shock*

Cells were also exposed to more severe heat shock at 42°C. For the tRNA$^{LysUUU}$ family, there is relatively no change in tRNA$^{LysUUU}$ intron levels when cells are exposed to a 42°C environment as opposed to cells grown at the permissive temperature of 23°C (Fig. 4A). Similarly, no change is observed in linear or circular intron levels when probing for the tRNA$^{TrpCCA}$ intron family (Fig. 4B). Astonishingly, the tRNA$^{ProUGG}$ intron accumulates readily upon shift to a 42°C environment, and the predominant intron band migrates as a different size than the predominant intron band observed in the 0 minute and 120 minute mock treatment lanes (Fig. 4C). This particular tRNA family consists of tRNAs with three different, naturally-occurring intron lengths, and thus, heat shock could affect the specificity of each tRNA$^{ProUGG}$ intron's degradation. A slight decrease in free tRNA$^{IleUAU}$ intron is observed upon 42°C heat shock to cells, but further investigation is needed to verify this result (Fig. 4D). While initial experiments could not confirm a 37°C temperature effect on tRNA intron turnover, 42°C heat

shock present an unanticipated effect. It remains possible that additional tRNA families could show tRNA intron accumulation such as that exhibited by $tRNA^{ProUGG}$.

*Analysis of oxidative stress*

Upon exposure of cells to media containing 3mM hydrogen peroxide, I observe a dramatic increase in the level of free $tRNA^{TrpCCA}$ linear intron (Fig. 5A). Within 30 minutes in the stress-containing media, tRNA transcription is inhibited as evidenced by the decreased levels of $tRNA^{TrpCCA}$ initial transcript (P) and the end-processed, intron-containing unspliced precursor (I) species. As anticipated, the level of free intron prior to stress exposure and in the 120 minute mock treatment is nearly undetectable. Remarkably, however, there are heightened quantities of the linear $tRNA^{TrpCCA}$ intron detected at the earliest time point measured (30 min) and there is no evidence of $tRNA^{TrpCCA}$ intron decay even after 120 minutes of exposure to oxidative stress. In contrast to accumulation of the linear $tRNA^{TrpCCA}$ free intron, the circular form of the $tRNA^{TrpCCA}$ intron does not accumulate in response to hydrogen peroxide stress. Less dramatic increases in free $tRNA^{IleUAU}$ intron and free $tRNA^{LysUUU}$ intron are observed upon exposure to oxidative stress, as shown in Fig. 5B,C, but additional experiments are needed to confirm if these increases are significant. In contrast to the linear form of the $tRNA^{TrpCCA}$ free intron and the $tRNA^{IleUAU}$ free intron, the other tRNA families probed under oxidative stress, specifically $tRNA^{LeuCAA}$, $tRNA^{ProUGG}$, $tRNA^{PheGAA}$, $tRNA^{SerCGA}$, and $tRNA^{LeuUAG}$, show no evidence of accumulation compared to unstressed cells (data not shown). The smaller free introns, including $tRNA^{PheGAA}$, $tRNA^{SerCGA}$, and $tRNA^{LeuUAG}$ are difficult to detect under any laboratory conditions, and thus we could not be certain of any changes in free intron levels.

*Analysis of ethanol stress*

The levels of $tRNA^{IleUAU}$, $tRNA^{TrpCCA}$, and $tRNA^{LysUUU}$ introns were observed in media containing 10% ethanol (Fig. 6). I observe that the levels of $tRNA^{IleUAU}$ decrease between 30 minutes and 120 minutes of exposure to stress, while the two control lanes, one isolated at 0 minutes and another after 120 minutes of growth in the absence of 10% EtOH, contain higher levels of intron (Fig. 6A). Similarly, $tRNA^{LysUUU}$ intron levels appear to decrease upon exposure to stressed media while the control lanes contain relatively higher amounts of intron (Fig. 6B). However, levels of the P and I species are also decreased upon ethanol treatment, suggesting ethanol may decrease $tRNA^{IleUAU}$ and $tRNA^{LysUUU}$ transcription. These experiments were completed only once, so the significance of these results cannot be concluded until additional replicates are obtained. No change in linear or circular intron levels was observed during the 120 minute time course for $tRNA^{TrpCCA}$ (Fig. 6C) Therefore, ethanol stress appears to have no effect the turnover of $tRNA^{TrpCCA}$. It remains unclear whether ethanol stress affects the turnover of free $tRNA^{IleUAU}$ or $tRNA^{LysUUU}$ introns. Still, it is possible that this stress could affect turnover of the introns derived from the other 7 untested tRNA families.

*Analysis of osmotic stress*

The levels of $tRNA^{IleUAU}$ (Fig. 7A), $tRNA^{TrpCCA}$ (Fig. 7B), $tRNA^{LysUUU}$ (Fig. 7C), and $tRNA^{SerCGA}$ (data not shown) were observed before and after subjecting cells to 0.5M sodium chloride-containing media. The 120 minute time course does not reveal any changes in free intron levels for these four families of tRNAs. While the $tRNA^{IleUAU}$ intron appears to be present in greater amounts at every time point compared to the other tRNA intron families presented, this is the tRNA intron family that is most easily and obviously witnessed under normal conditions. It is more noteworthy that this tRNA's intron levels do not significantly change upon exposure to sodium chloride from the intron levels found in unstressed cells. Although my studies do not

uncover tRNA introns which accumulate or are degraded more rapidly under osmotic stress, the potential for these results to arise from untested tRNA families remains a possibility for future studies.

*Analysis of rapamycin stress*

Rapamycin stress fails to indicate any significant changes in free $tRNA^{IleUAU}$ (Fig. 8A), $tRNA^{TrpCCA}$ (Fig. 8B), or $tRNA^{LysUUU}$ (Fig. 8C) intron levels. Rapamycin stress does reveal a decrease in the levels of initial tRNA transcripts after 90 minutes of exposure to stress, for all three families shown. tRNAs are transcribed by RNA polymerase III, which is regulated by the target of rapamycin (TOR) pathway. Thus, the transcription of new tRNAs is affected by the growth of cells in presence of rapamycin. The decrease in initial transcript levels translates to a minor dip in $tRNA^{IleUAU}$ and $tRNA^{TrpCCA}$ intron levels after 90 minutes of exposure to rapamycin stress (Fig. 8 A,B). Ultimately, these experiments need to be replicated in order to determine if this observation is repeatable and significant. In addition, the remaining 7 tRNA families need to be probed for in order to observe these families' tRNA intron levels when cells are subjected to rapamycin stress.

*Analysis of glucose deprivation*

Upon depriving cells of glucose, the levels of $tRNA^{IleUAU}$ intron gradually decrease with time (Fig. 9A). After 120 minutes of glucose starvation, this decrease in $tRNA^{IleUAU}$ intron levels is significant compared to the higher level of free intron in the 120 minute mock treatment group, which was grown in media containing glucose. Interestingly, this decrease in free intron is not observed in combination with a decrease in the transcription of new tRNAs, as shown by the steady levels of $tRNA^{TrpCCA}$ initial transcript and end-processed, intron-containing unspliced precursor species. The gradual decrease in $tRNA^{IleUAU}$ intron levels is observed in multiple,

experimental replicates, suggesting a consistent effect on wild-type yeast cells. On the other hand, this observation is not reiterated by $tRNA^{Trp}_{CCA}$ (Fig. 9B), $tRNA^{Lys}_{UUU}$ (Fig. 9C), $tRNA^{Leu}_{CAA}$, $tRNA^{Pro}_{UGG}$, or $tRNA^{Ser}_{GCU}$ (data not shown). Instead, these tRNA families show steady levels of very little free tRNA intron. It is important to keep in mind that tRNA introns are normally degraded very quickly when cells are not exposed to stress, and thus not all intron bands are clearly visible. Rather, the result seen for the $tRNA^{Ile}_{UAU}$ family is noteworthy because the introns are turned over at an accelerated rate when the cells are starved for glucose, which is evidenced by the difference in intron levels in the 120 minute glucose deprived lane and the 120 minute mock treatment lane.

*Analysis of saturated growth stress*

Analysis of tRNA introns under saturated growth conditions reveals that the $tRNA^{Ile}_{UAU}$ intron is considerably more stable during log phase than other tRNA families, and its degradation does not begin until saturated growth is reached (Fig. 10A). The $tRNA^{Ile}_{UAU}$ intron is visible from 0.3-1.3 $OD_{600}$. This is observation is consistent with the decrease in $tRNA^{Ile}_{UAU}$ intron levels seen in glucose deprivation conditions. As wild-type cells reach saturated growth conditions, glucose becomes exhausted and the $tRNA^{Ile}_{UAU}$ intron is more rapidly degraded, signifying a limited need for the free $tRNA^{Ile}_{UAU}$ intron when cell growth is saturated. This experiment also verifies that free $tRNA^{Trp}_{CCA}$ and $tRNA^{Lys}_{UUU}$ introns are rarely detected under laboratory conditions (Fig. 10B,C). Because cells used in this experiment were not exposed to a particular stress in the growth media and were simply grown to saturation, the quick rate of tRNA intron turnover is seen. Moreover, observing this quick rate of intron turnover highlights the impact of stress conditions which alter tRNA intron turnover.

## DISCUSSION

### *tRNA family-specific stress-specific tRNA intron accumulation*

I show that tRNA introns accumulate in a family-specific, stress-specific manner. I most obviously see $tRNA^{TrpCCA}$ linear introns accumulate under oxidative stress, $tRNA^{ProUGG}$ introns accumulate under 42°C heat shock, and $tRNA^{IleUAU}$ introns more rapidly degraded under glucose starvation. The differences in the impact on each tRNA family in each stress condition highlights the complexity of the tRNA intron turnover process, as well as the biological significance of tRNA introns. These studies represent the conditions to which yeasts in nature are regularly exposed, and offer an approach to understanding how tRNA intron turnover proceeds in wild-type cells, outside of strict laboratory conditions.

### *Potential mechanisms of altering tRNA intron degradation*

The observed accumulation and/or accelerated degradation of certain tRNA introns under stress could be explained by multiple potential mechanisms. First, the stress to the cell could directly alter the proteins that function in the turnover of free introns. These proteins could include the kinase Rlg1, and the exonuclease Xrn1, which are both known to be involved in the turnover of $tRNA^{IleUAU}$ and $tRNA^{LeuCAA}$. However, one would expect tRNA introns turned over by the same enzymes to have similar responses. I instead report different responses of $tRNA^{TrpCCA}$, and $tRNA^{ProUGG}$ under 42°C heat shock conditions (Fig. 4B,C). Both of these tRNA families have intron turnover mechanisms dependent on Xrn1 and independent of Rlg1. Moreover, $tRNA^{LysUUU}$ and $tRNA^{IleUAU}$ introns are also degraded by Xrn1, but they do not accumulate when cells are exposed to 42°C heat shock conditions (Fig. 4A,D). Thus, if heat was affecting Xrn1 or its location, the four intron families should display the same patterns of

accumulation. Furthermore, the stress condition could be affecting unidentified proteins in the turnover process.

The environmental stress could also be indirectly affecting the enzymes involved in tRNA intron turnover by altering their subcellular locations or their catalytic activity. A change in catalytic activity would suggest additional kinases and phosphatases involved in the activation and inhibition of the proteins that function directly in intron turnover. Additionally, the stress could promote the localization of free introns to stress granules or other ribonuclear protein granules. Stress granule formation is known to be induced by interactions between non-translating RNAs, making tRNA introns a noteworthy candidate for this process (Protter and Parker, 2016). Another hypothesis is that the timing of degradation of tRNA introns is dependent on the length of the intron. The $tRNA^{IleUAU}$ family, in particular, is most often and easily visible by Northern blotting, while others are almost never detected, including $tRNA^{LeuUAG}$, $tRNA^{SerCGA}$, and $tRNA^{TyrGUA}$. The tRNA introns that are more easily visualized tend to be the longer ones. While $tRNA^{IleUAU}$ has an intron of 60 nucleotides and $tRNA^{TrpCCA}$ has an intron of 34 nucleotides, $tRNA^{LeuUAG}$, and $tRNA^{SerCGA}$ contain introns of 19 nucleotides, and $tRNA^{TyrGUA}$ has an intron of only 14 nucleotides. The differences in the lengths of the introns may contribute to how rapidly the exonuclease is able to degrade them, or how accessible they are to the kinase.

*Potential function of tRNA introns*

The Hopper lab has helped to illuminate the multiplicity and importance of the tRNA intron turnover pathway. Our data suggests that tRNA introns serve a potential physiological function. A previous member of the Hopper lab, Alicia Bao, used Pattern Match, a search engine on the Saccharomyces Genome Database, to identify mRNA targets complementary to tRNA introns. She was able to identify numerous genes with long stretches of perfect complementation

to tRNA introns, the longest being 15 nucleotides. The probability of this length of complementarity happening by chance in the yeast genome is 1 in 1.1 billion, indicating high unlikelihood of this complementation being coincidental. Her searches, along with the identification of conditions under which free introns remain intact in the cell, led us to speculate on the potential role of tRNA introns in gene regulation. Another lab member, Dr. Gina Nostramo, began investigating the hypothesis that tRNA introns function similarly to small, non-coding regulatory RNAs. She termed the short regions of complementarity in the yeast genome TIC sequences (tRNA intron complementary sequences). Her focus has been on a few particular genes, including *GPM2* and *ATG5*, selected for their complementation to tRNA families with known methods to manipulate the tRNA intron levels. Using a $tRNA_{IleUAU}$ intron deletion strain, Gina observes increased levels of *GPM2* mRNA and protein. Furthermore, the phenotype of increased mRNA levels can be rescued upon addition of a single copy of the $tRNA_{IleUAU}$-1 gene. Interestingly, the $tRNA_{TrpCCA}$ intron has two 14 base pair regions of complementarity to *ATG5*, a gene involved in autophagy. Because the $tRNA_{TrpCCA}$ linear intron accumulates so readily under oxidative stress, Gina is able to observe the impact of increased intron levels on this gene. Upon hydrogen peroxide treatment, $tRNA_{TrpCCA}$ linear intron levels increase, and *ATG5* mRNA levels decrease. Further experiments are being conducted to confirm that these observations are a result of the interactions between mRNA and tRNA intron.

With my studies, we hope to continue to uncover stress conditions under which tRNA intron turnover is affected, and interactions of free tRNA introns in the cell be discovered as an attempt to understand why tRNA introns have been conserved. My studies need to be expanded to all 10 families of intron-containing tRNAs. This process will be able to be completed efficiently with the creation of probe mixtures containing oligos for four different intron

sequences for tRNA introns of varying lengths, by another Hopper lab member, Sara Metcalf. This improved efficiency will allow my studies to be expanded to additional stress conditions as well. The discovery of new stress conditions under which other tRNA introns are affected will also allow for the expansion of Gina's work to screen additional genes with TIC sequences to these other tRNA introns. Eventually, we hope to be able to understand why tRNA intron turnover is such an important process and why tRNA introns have been maintained through evolution.

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**REFERENCES**


1. Chan, P. P., & Lowe, T. M. (2009). GtRNAdb: A database of transfer RNA genes detected in genomic sequence. Nucleic Acids Research, 37, 93–97. https://doi.org/10.1093/nar/gkn787

2. Chan, P. P., & Lowe, T. M. (2016). GtRNAdb 2.0: An expanded database of transfer RNA genes identified in complete and draft genomes. Nucleic Acids Research, 44, D184–D189. https://doi.org/10.1093/nar/gkv1309

3. Czech, A., Wende, S., Mörl, M., Pan, T., & Ignatova, Z. (2013). Reversible and rapid transfer-RNA deactivation as a mechanism of translational repression in stress. *PLoS genetics*, *9*(8), e1003767. https://doi.org/10.1371/journal.pgen.1003767

4. Hayashi, S., Mori, S., Suzuki, T., Suzuki, T., & Yoshihisa, T. (2019). Impact of intron removal from tRNA genes on Saccharomyces cerevisiae. *Nucleic acids research*, *47*(11), 5936–5949. https://doi.org/10.1093/nar/gkz270

5. Hopper A. K. (2013). Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast Saccharomyces cerevisiae. *Genetics*, *194*(1), 43–67. https://doi.org/10.1534/genetics.112.147470

6. Hou, Y. M., & Yang, X. (2013). Regulation of cell death by transfer RNA. *Antioxidants & redox signaling*, *19*(6), 583–594. https://doi.org/10.1089/ars.2012.5171

7. Huang, S. Q., Sun, B., Xiong, Z. P., Shu, Y., Zhou, H. H., Zhang, W., Xiong, J., & Li, Q. (2018). The dysregulation of tRNAs and tRNA derivatives in cancer. *Journal of experimental & clinical cancer research : CR*, *37*(1), 101. https://doi.org/10.1186/s13046-018-0745-z

8. Ivanov, P., Emara, M. M., Villen, J., Gygi, S. P., & Anderson, P. (2011). Angiogenin-induced tRNA fragments inhibit translation initiation. *Molecular cell*, *43*(4), 613–623. https://doi.org/10.1016/j.molcel.2011.06.022

9. Kim, H. K., Fuchs, G., Wang, S., Wei, W., Zhang, Y., Park, H., Roy-Chaudhuri, B., Li, P., Xu, J., Chu, K., Zhang, F., Chua, M. S., So, S., Zhang, Q. C., Sarnow, P., & Kay, M. A. (2017). A transfer-RNA-derived small RNA regulates ribosome biogenesis. *Nature*, *552*(7683), 57–62. https://doi.org/10.1038/nature25005

10. Lu, Z., Filonov, G. S., Noto, J. J., Schmidt, C. A., Hatkevich, T. L., Wen, Y., ... Matera, A. G. (2015). Metazoan tRNA introns generate stable circular RNAs in vivo. RNA, 21, 1554–1565. https://doi.org/10.1261/rna.052944.115

11. Protter, D., & Parker, R. (2016). Principles and Properties of Stress Granules. *Trends in cell biology*, *26*(9), 668–679. https://doi.org/10.1016/j.tcb.2016.05.004

12. Richards, A. L., Watza, D., Findley, A., Alazizi, A., Wen, X., Pai, A. A., Pique-Regi, R., & Luca, F. (2017). Environmental perturbations lead to extensive directional shifts in RNA processing. *PLoS genetics*, *13*(10), e1006995. https://doi.org/10.1371/journal.pgen.1006995

13. Thompson, D. M., Lu, C., Green, P. J., & Parker, R. (2008). tRNA cleavage is a conserved response to oxidative stress in eukaryotes. *RNA (New York, N.Y.)*, *14*(10), 2095–2103. https://doi.org/10.1261/rna.1232808

14. Wan, Y., & Hopper, A. K. (2018). From powerhouse to processing plant: conserved roles of mitochondrial outer membrane proteins in tRNA splicing. *Genes & development*, *32*(19-20), 1309–1314. https://doi.org/10.1101/gad.316257.118

15. Wu, J., Bao, A., Chatterjee, K., Wan, Y., & Hopper, A. K. (2015). Genome-wide screen uncovers novel pathways for tRNA processing and nuclear-cytoplasmic dynamics. *Genes & development*, *29*(24), 2633–2644. https://doi.org/10.1101/gad.269803.115

16. Wu, J., & Hopper, A. K. (2014). Healing for destruction: tRNA intron degradation in yeast is a two-step cytoplasmic process catalyzed by tRNA ligase Rlg1 and 5'-to-3' exonuclease Xrn1. *Genes & development*, *28*(14), 1556–1561. https://doi.org/10.1101/gad.244673.114

17. Wu, J., Huang, H. Y., & Hopper, A. K. (2013). A rapid and sensitive non-radioactive method applicable for genome-wide analysis of Saccharomyces cerevisiae genes involved in small RNA biology. *Yeast (Chichester, England)*, *30*(4), 119–128. https://doi.org/10.1002/yea.2947

18. Zhang, Z., Ye, Y., Gong, J., Ruan, H., Liu, C. J., Xiang, Y., Cai, C., Guo, A. Y., Ling, J., Diao, L., Weinstein, J. N., & Han, L. (2018). Global analysis of tRNA and translation factor expression reveals a dynamic landscape of translational regulation in human cancers. *Communications biology*, *1*, 234. https://doi.org/10.1038/s42003-018-0239-8

## 1. TABLES AND FIGURES

| Oligo name | Sequence | Description |
|---|---|---|
| SRIM03 | 5' CGTTGCTTTTAAAGGCCTGTTTGAAAGGTCTTTGGCACAGAAACTTCGGAAACCGAATGTTGCTAT 3' | intron probe for $tRNA_{IleUAU}$ |
| JW0044 | 5' TATTCCCACAGTTAACTGCGGTCAAGATATTT 3' | intron probe for $tRNA_{LeuCAA}$ |
| JW0056 | 5' TGCTTTGTCTTCCTGTTTAATCAGGAAGTCG 3' | intron probe for $tRNA_{ProUGG}$ |
| JW0057 | 5' TGCAATCTTATTCCGTGGAATTTCCAAGATTTAA 3' | intron probe for $tRNA_{TrpCCA}$ |
| JW0058 | 5' ATC CTT GCT TAA GCA AAT GCG CT -3' | intron probe for $tRNA_{LysUUU}$ |
| AB01 | 5' AACTTGACCGAAGTTTTTT 3' | intron probe for $tRNA_{PheGAA}$ |
| AB02 | 5' AGCCGAACTTTTTATTCCA 3' | intron probe for $tRNA_{SerCGA}$ |
| AB03 | 5' AATTGCTTTTCTGAGGAAA 3' | intron probe for $tRNA_{SerGCU}$ |
| AB05 | 5' TTCGTAGTGATAAA 3' | intron probe for $tRNA_{TyrGUA}$ |
| AB06 | 5' ATTTTAGAGGTTAAATCCA 3' | intron probe for $tRNA_{LeuUAG}$ |

**Table 1:** Oligonucleotides

| Stress Condition | Dependent on both Xrn1 and Rlg1 | | | Dependent mainly on Xrn1 | | | Dependent mainly on Rlg1 | | Independent of both Xrn1 and Rlg1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Ile_{UAU}$ | $Leu_{CAA}$ | $Lys_{UUU}$ | $Trp_{CCA}$ | $Trp_{CCA}$ (circular) | $Pro_{UGG}$ | $Phe_{GAA}$ | $Ser_{GCU}$ | $Ser_{CGA}$ | $Leu_{UAG}$ | $Tyr_{GUA}$ |
| 37°C | | ↓ | - | | | | | | | | |
| 42°C | - | | - | - | - | ↑↑ | | | | | |
| 3mM $H_2O_2$ | ↑ | - | ↑ | ↑↑ | - | - | - | | - | - | |
| 10% EtOH | ↓ | | ↓ | - | - | | | | | | |
| 0.5M NaCl | - | | - | - | - | | | | - | | |
| Rapamycin | - | | - | - | - | | | | | | |
| - glucose | ↓↓ | | - | - | - | | | - | | | |
| Saturation | ↓ | | - | - | - | | | | | | |

**Table 2:** Stresses and tRNA families tested. A single arrow down indicates an accelerated degradation of tRNA introns observed under stress, but not replicated nor statistically significant data. A single arrow up indicates the accumulation of tRNA introns observed under stress, but not replicated nor statistically significant data. Two bold arrows down indicate statistically significant data of accelerated tRNA intron turnover under stress. Two bold arrows up indicate statistically significant data of tRNA intron accumulation under stress. A dash indicates no change in tRNA intron turnover upon stress. A blank cell indicates not yet tested.

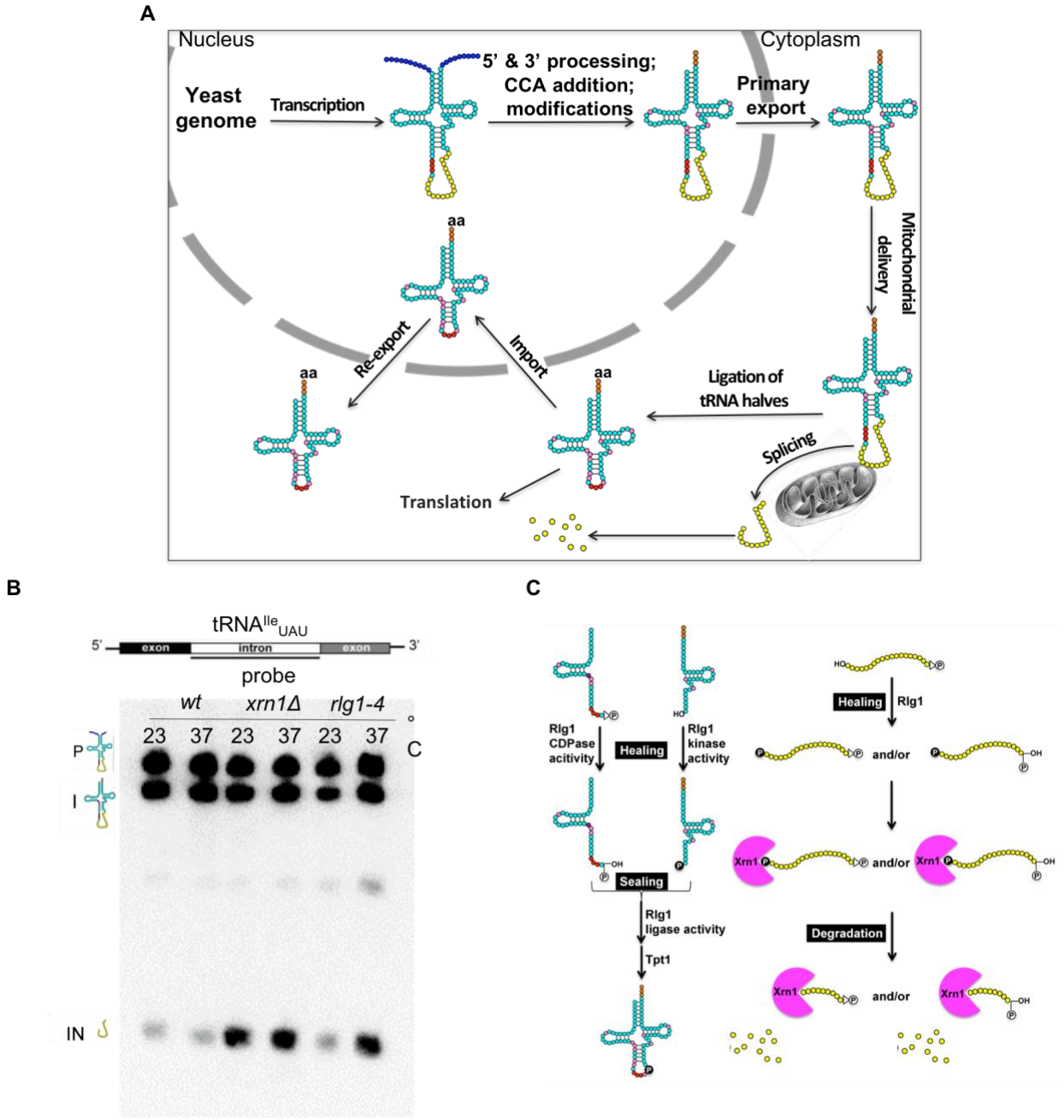



**Figure 1:** tRNA trafficking in yeast and intron turnover steps uncovered by the Hopper lab. **(A)** Schematic representation of tRNA processing steps in yeast. **(B)** $tRNA_{IleUAU}$ introns accumulate in *xrn1Δ* and *rlg1-4* mutant strains, as shown by Northern blotting. Schematic representation of $tRNA_{IleUAU}$ intron turnover mechanism. Upon release from the tRNA molecule, the free intron is phosphorylated on the 5' end by Rlg1, and then Xrn1 degrades the intron in the 5' to 3' direction, resulting in free nucleotides. Figure taken from Wu and Hopper 2014.

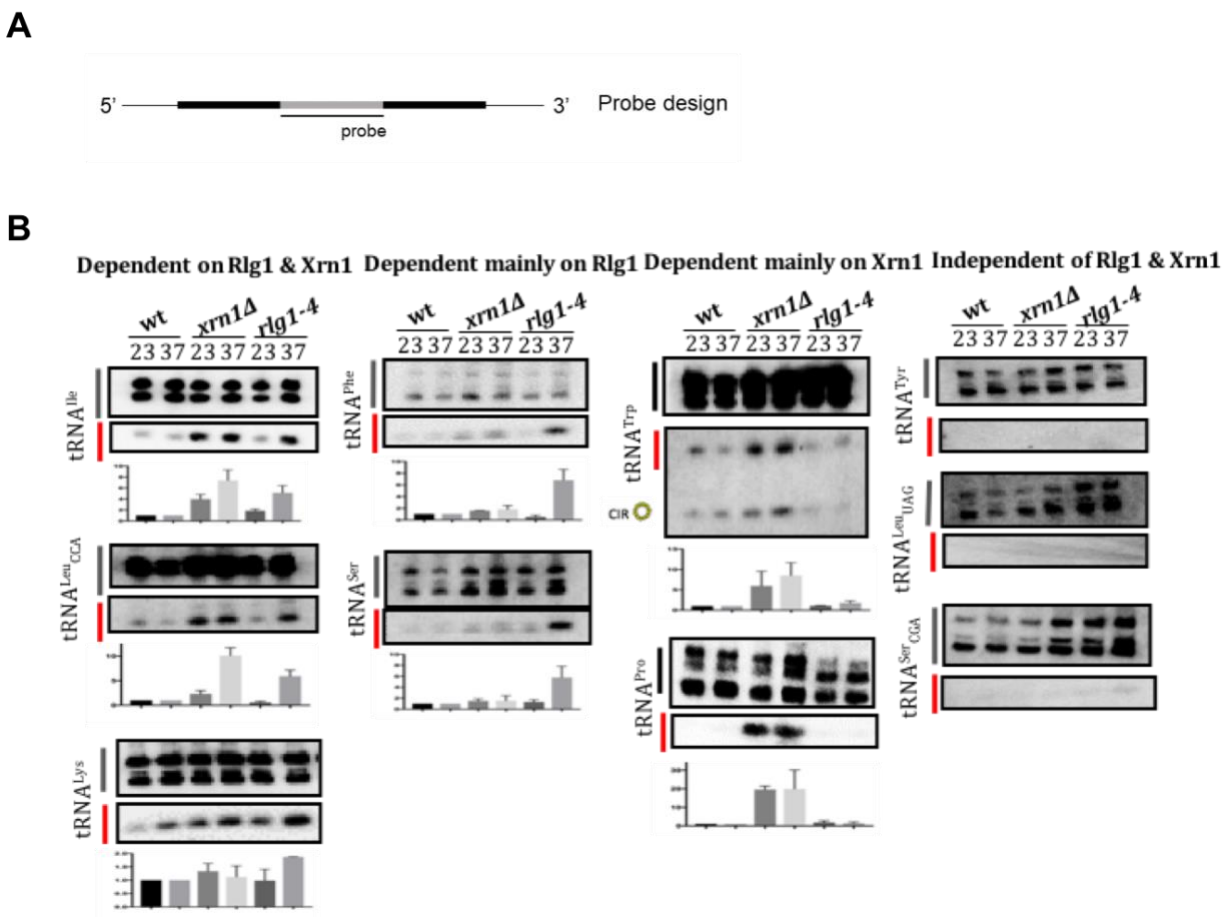



**Figure 2:** Classification of all ten intron-containing tRNA families by intron turnover mechanism. **(A)** Representation of the non-radioactive DIG-labeled probe hybridized to each tRNA intron that was used to detect intron levels. **(B)** Each intron-containing tRNA family is grouped into one of four categories based on relative tRNA intron levels observed in wt, *xrn1Δ*, and *rlg1-4* cells. The first column includes $tRNA^{IleUAU}$, $tRNA^{LeuCAA}$, and $tRNA^{LysUUU}$, which have intron turnover mechanisms dependent on both Xrn1 and Rlg1, as evidenced by the tRNA intron accumulation in both mutants relative to wt. The second column includes $tRNA^{PheGAA}$ and $tRNA^{SerGCU}$, which have intron turnover mechanisms dependent mainly on Rlg1, as more tRNA intron accumulation is seen in the *rlg1-4* mutant. The third column, including $tRNA^{TrpCCA}$ and $tRNA^{ProUGG}$, is dependent mainly on Xrn1 for intron turnover, as more tRNA intron accumulation is observed in the *xrn1*Δ mutant. The fourth column shows no tRNA intron accumulation in either the *xrn1*Δ mutant or the *rlg1-4* mutant, suggesting that tRNA intron turnover is independent of both Xrn1 and Rlg1, and includes the $tRNA^{TyrGUA}$, $tRNA^{LeuUAG}$, and $tRNA^{SerCGA}$ families. All quantification is normalized to the wt intron level. All Northern blots and quantification done by Alicia Bao.

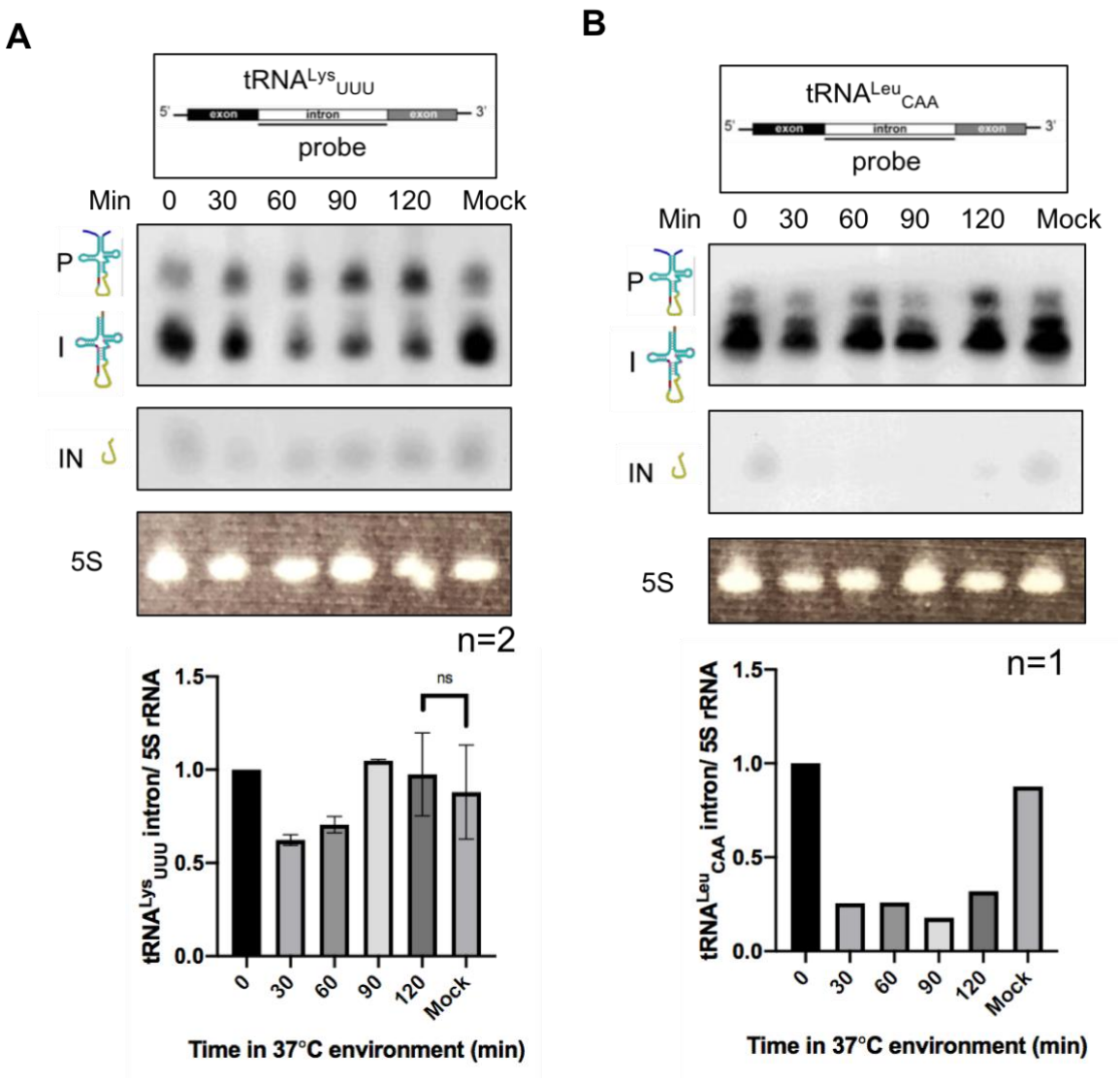
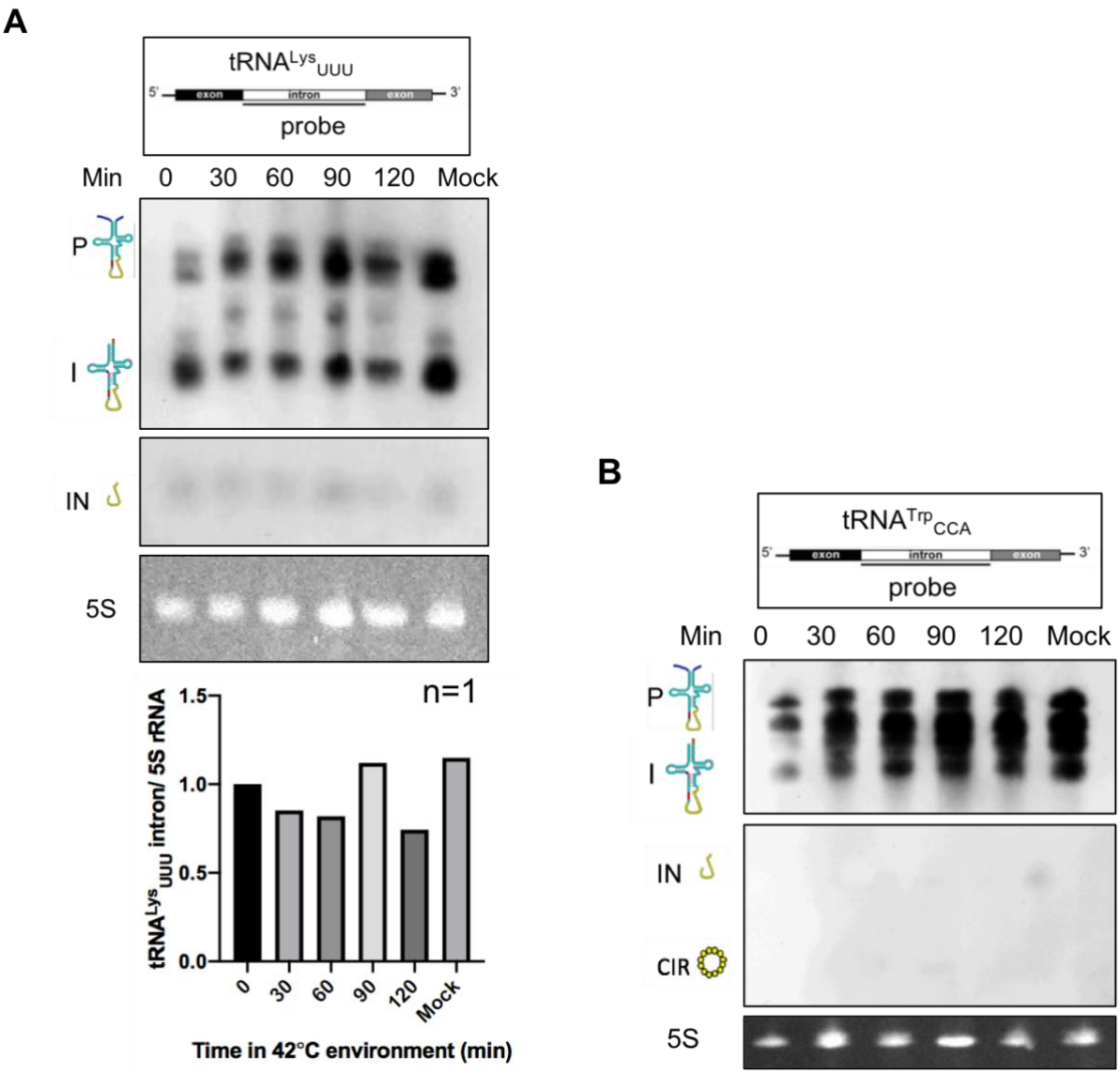



**Figure 3:** 37°C temperature stress does not show significant changes in tRNA intron levels. **(A)** tRNA$_{LysUUU}$ intron exhibits no significant change in turnover upon exposure to temperature stress. **(B)** tRNA$_{LeuCAA}$ shows a decrease in tRNA intron levels upon exposure to temperature stress. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.


A
tRNA$^{Lys}_{UUU}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
5S
n=1
tRNA$^{Lys}_{UUU}$ intron/ 5S rRNA
1.5
1.0
0.5
0.0
0 30 60 90 120 Mock
Time in 42°C environment (min)
B
tRNA$^{Trp}_{CCA}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
CIR
5S

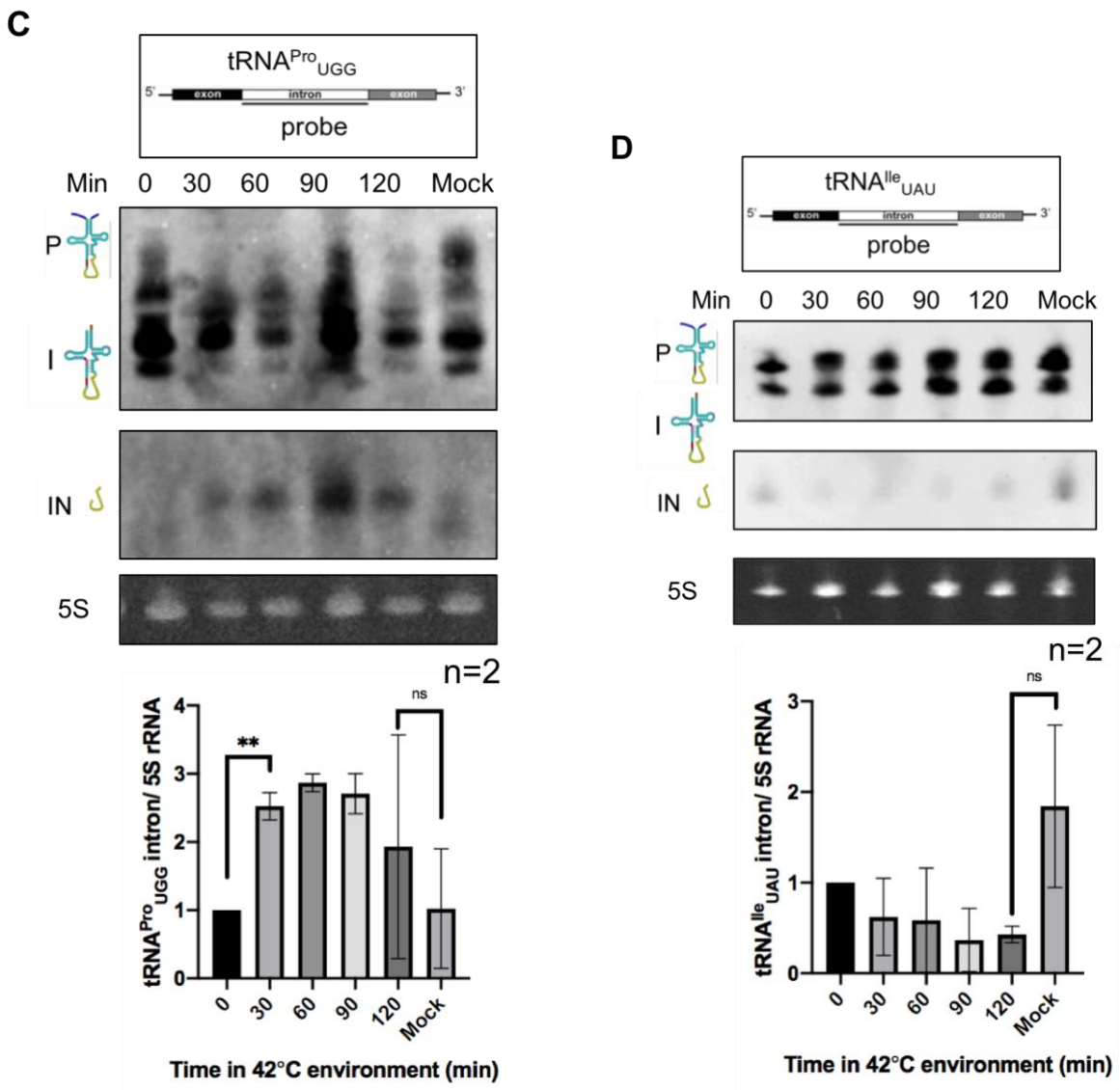
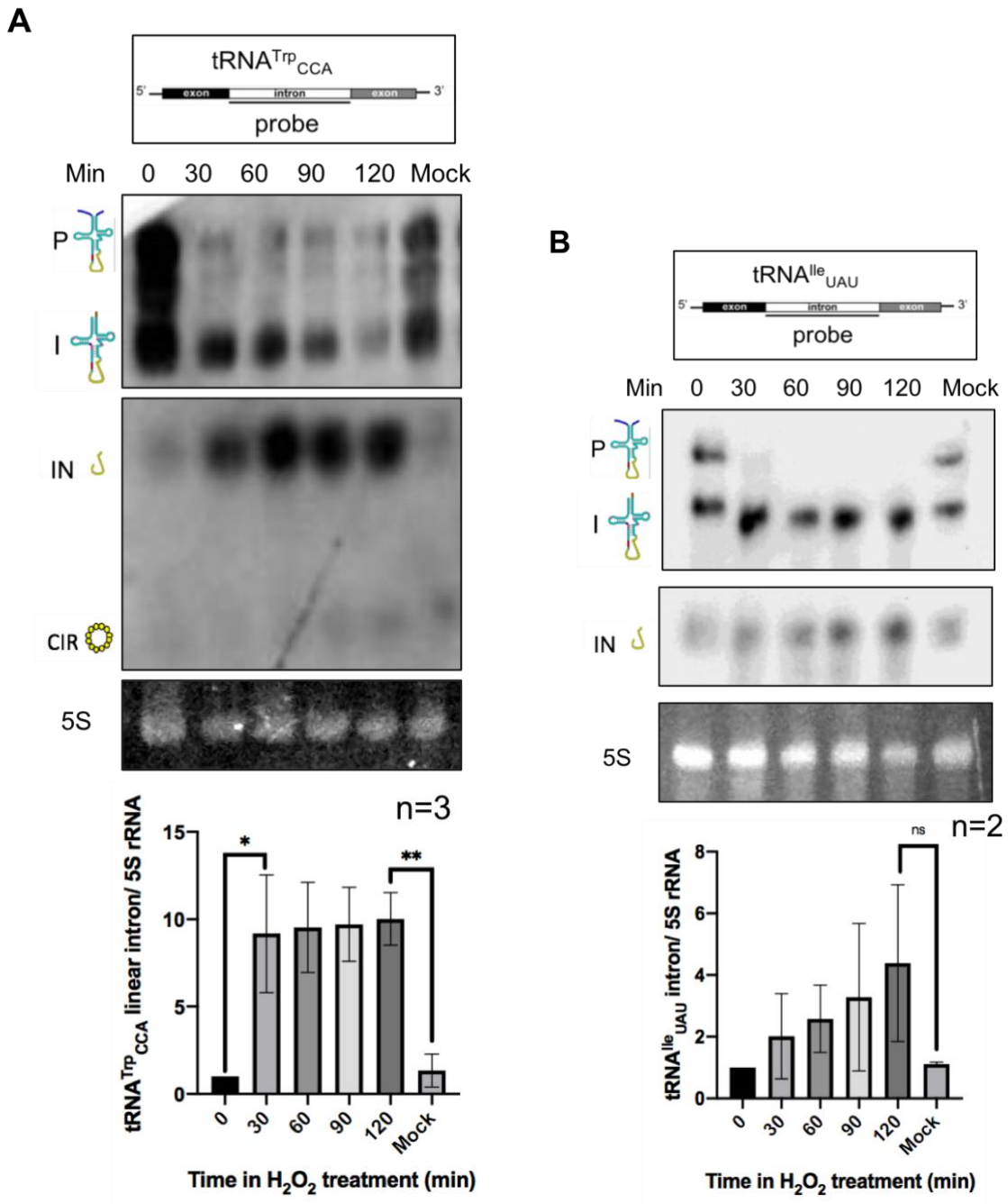



**Figure 4:** 42°C heat shock causes accumulation of tRNA$_{ProUGG}$ introns. **(A)** tRNA$_{LysUUU}$ intron does not accumulate upon heat shock to wt cells. **(B)** tRNA$_{TrpCCA}$ linear and circular intron levels show no change in a 42°C environment. **(C)** tRNA$_{ProUGG}$ intron accumulates under heat shock conditions. **(D)** tRNA$_{IleUAU}$ shows no significant change in tRNA intron levels upon heat shock. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.


A
tRNA$^{Trp}_{CCA}$
5' exon intron exon 3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
CIR
5S
n=3
tRNA$^{Trp}_{CCA}$ linear intron/ 5S rRNA
Time in $H_2O_2$ treatment (min)
B
tRNA$^{Ile}_{UAU}$
5' exon intron exon 3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
5S
n=2
ns
tRNA$^{Ile}_{UAU}$ intron/ 5S rRNA
Time in $H_2O_2$ treatment (min)

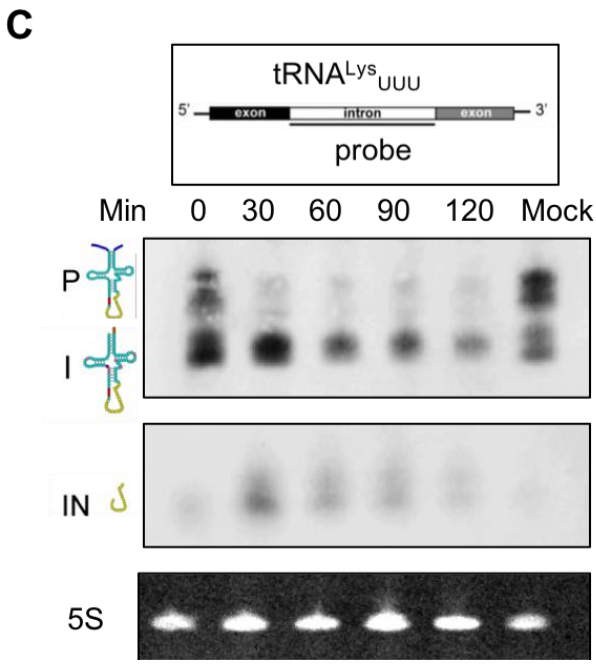



**Figure 5:** Oxidative stress causes significant accumulation of tRNA$^{TrpCCA}$ linear intron. **(A)** High levels of tRNA$^{TrpCCA}$ linear intron accumulate upon exposure to oxidative stress. No effect is seen on tRNA$^{TrpCCA}$ circular intron form. **(B)** Exposure to $H_2O_2$ shows an insignificant increase in tRNA$^{IleUAU}$ intron levels relative to untreated cells. **(C)** tRNA$^{LysUUU}$ intron probed on top of a blot probing for the tRNA$^{TrpCCA}$ intron. The top band in the intron row may indicate tRNA$^{LysUUU}$ intron accumulation upon oxidative stress. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.

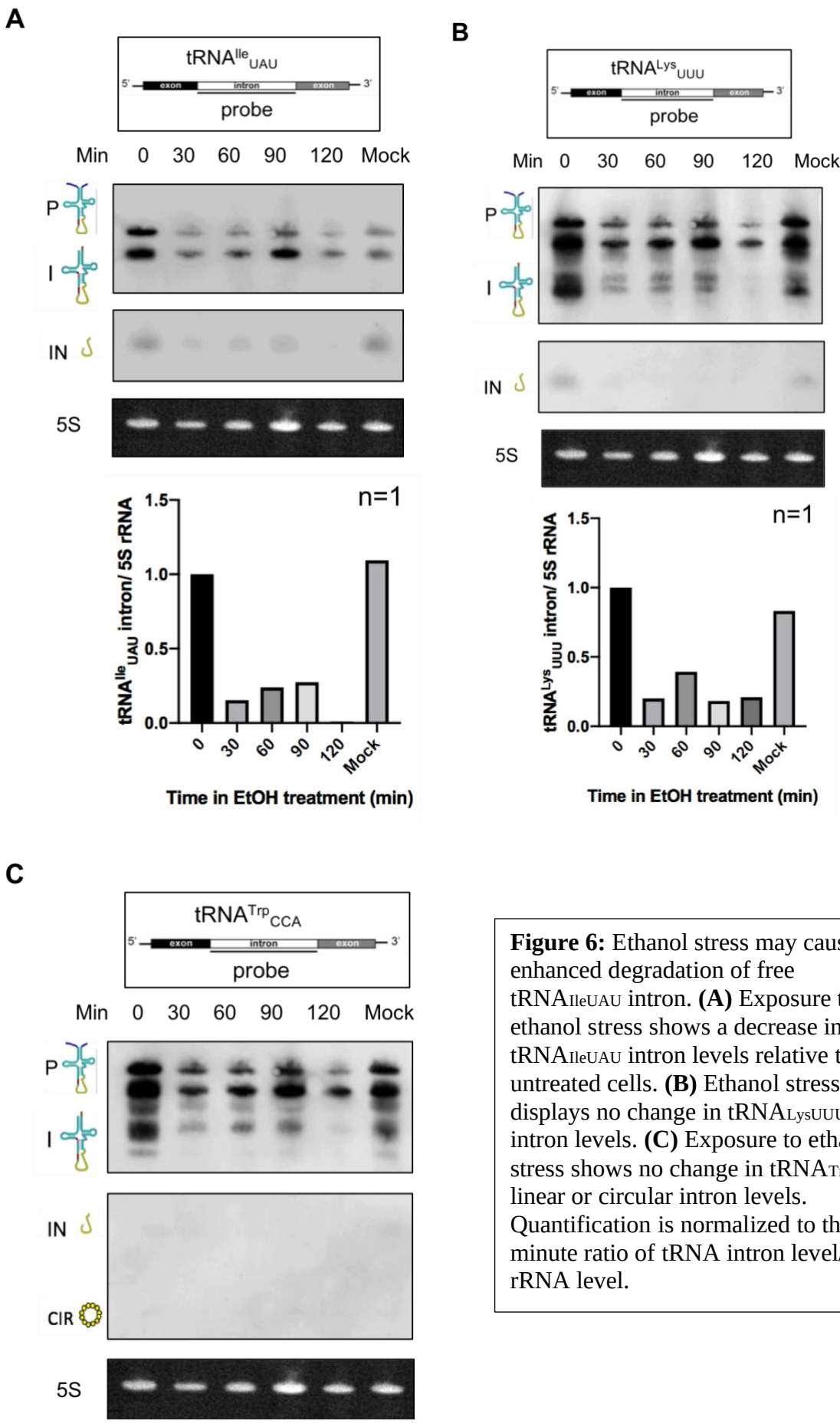
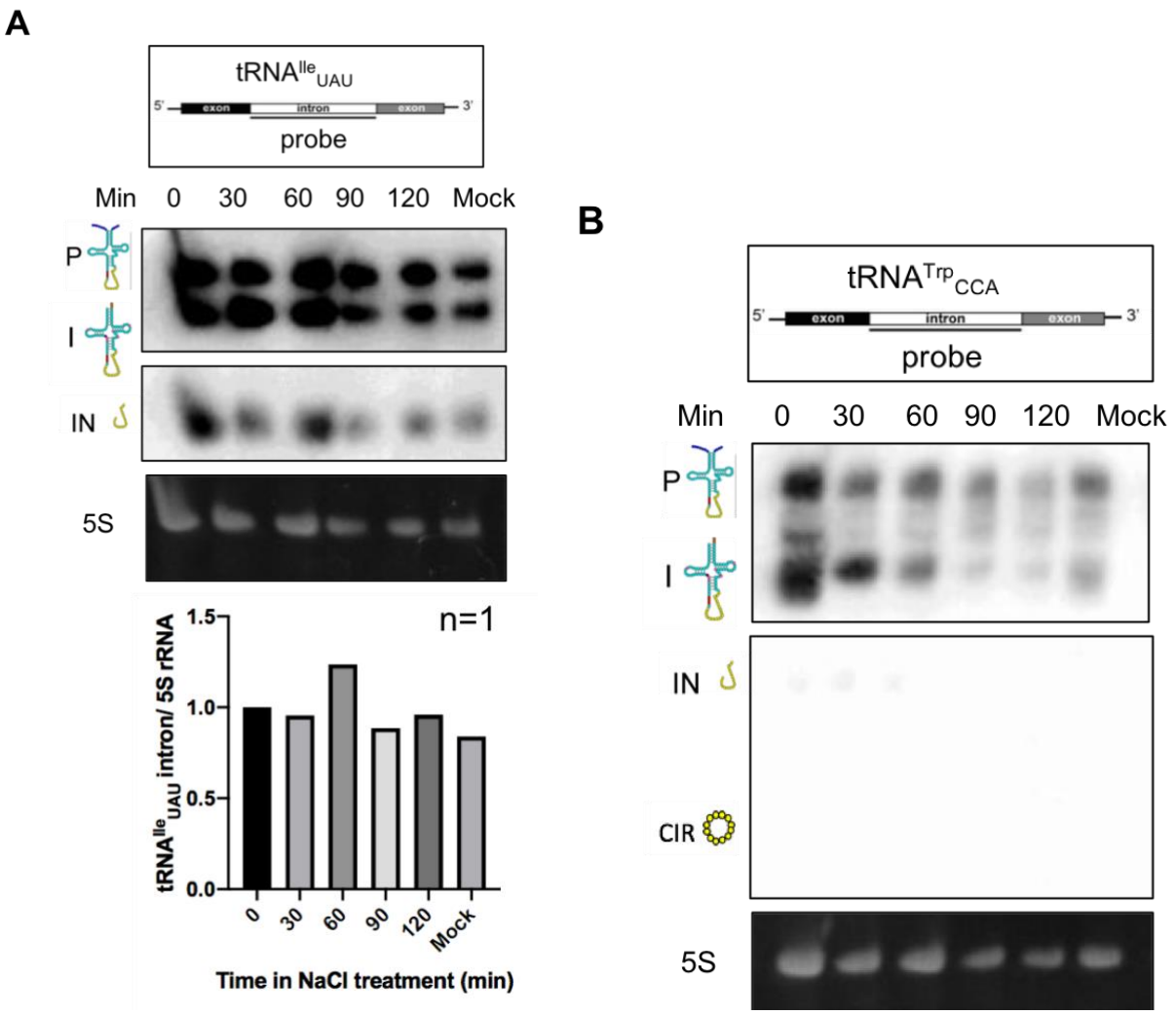



**Figure 6:** Ethanol stress may cause enhanced degradation of free $tRNA_{IleUAU}$ intron. **(A)** Exposure to ethanol stress shows a decrease in $tRNA_{IleUAU}$ intron levels relative to untreated cells. **(B)** Ethanol stress displays no change in $tRNA_{LysUUU}$ intron levels. **(C)** Exposure to ethanol stress shows no change in $tRNA_{TrpCCA}$ linear or circular intron levels. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.


A
tRNA$^{Ile}_{UAU}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
5S
n=1
tRNA$^{Ile}_{UAU}$ intron/ 5S rRNA
1.5
1.0
0.5
0.0
0 30 60 90 120 Mock
Time in NaCl treatment (min)
B
tRNA$^{Trp}_{CCA}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
CIR
5S

C

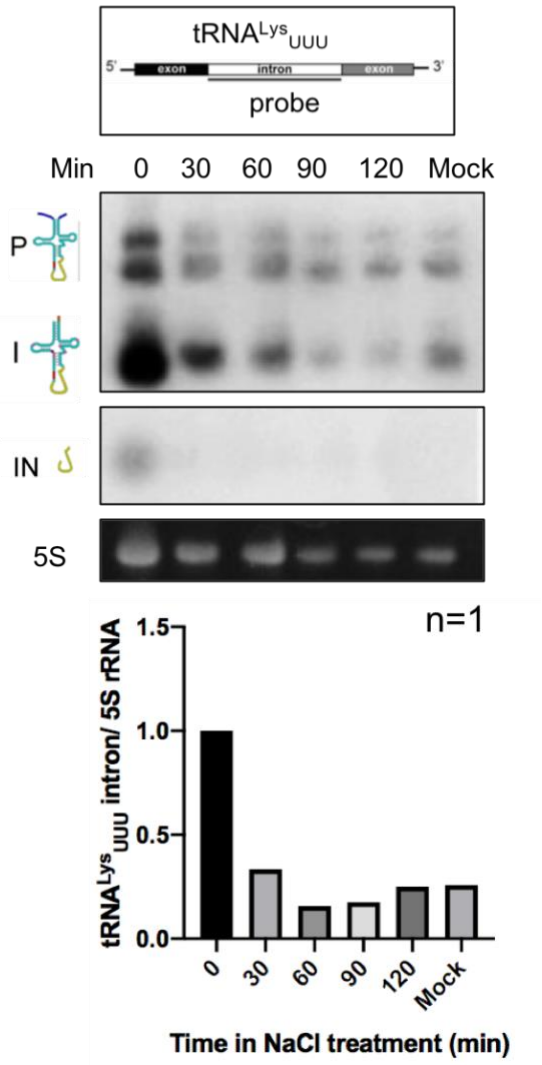
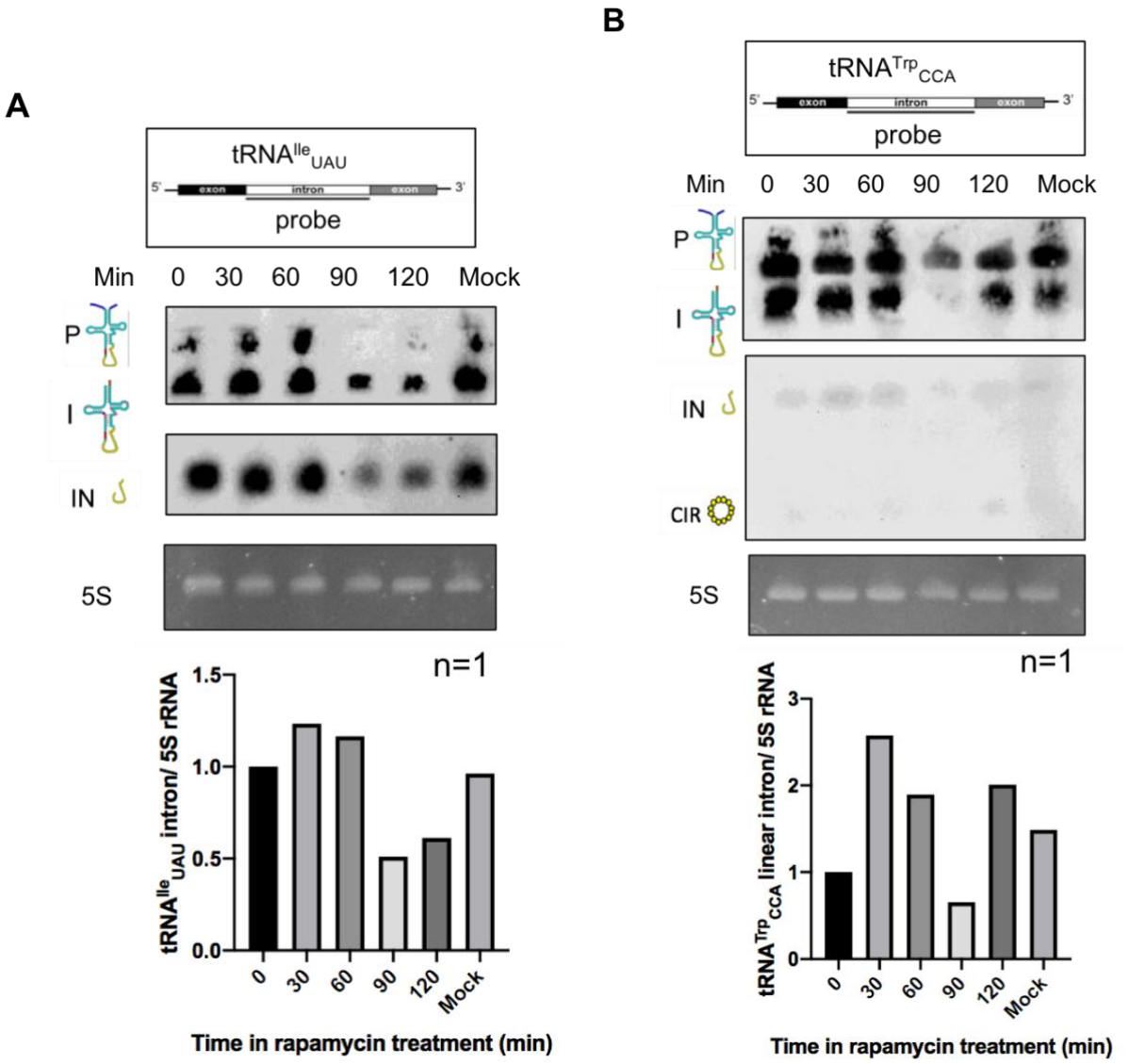



**Figure 7:** Osmotic stress does not alter tRNA free intron levels. **(A)** $tRNA_{IleUAU}$ intron levels do not change upon exposure to NaCl. **(B)** Osmotic stress shows no change in $tRNA_{TrpCCA}$ linear or circular intron levels. **(C)** $tRNA_{LysUUU}$ intron levels are not affected by exposure to NaCl. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.


A
tRNA$^{Ile}_{UAU}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
5S
n=1
tRNA$^{Ile}_{UAU}$ intron/ 5S rRNA
1.5
1.0
0.5
0.0
0 30 60 90 120 Mock
Time in rapamycin treatment (min)
B
tRNA$^{Trp}_{CCA}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
CIR
5S
n=1
tRNA$^{Trp}_{CCA}$ linear intron/ 5S rRNA
3
2
1
0
0 30 60 90 120 Mock
Time in rapamycin treatment (min)

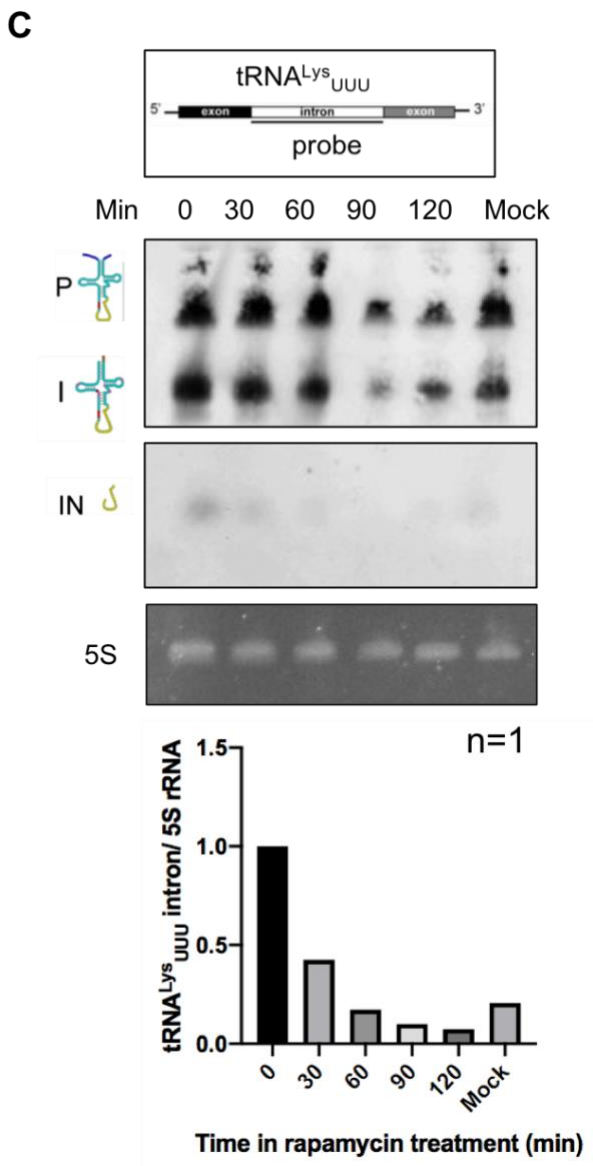
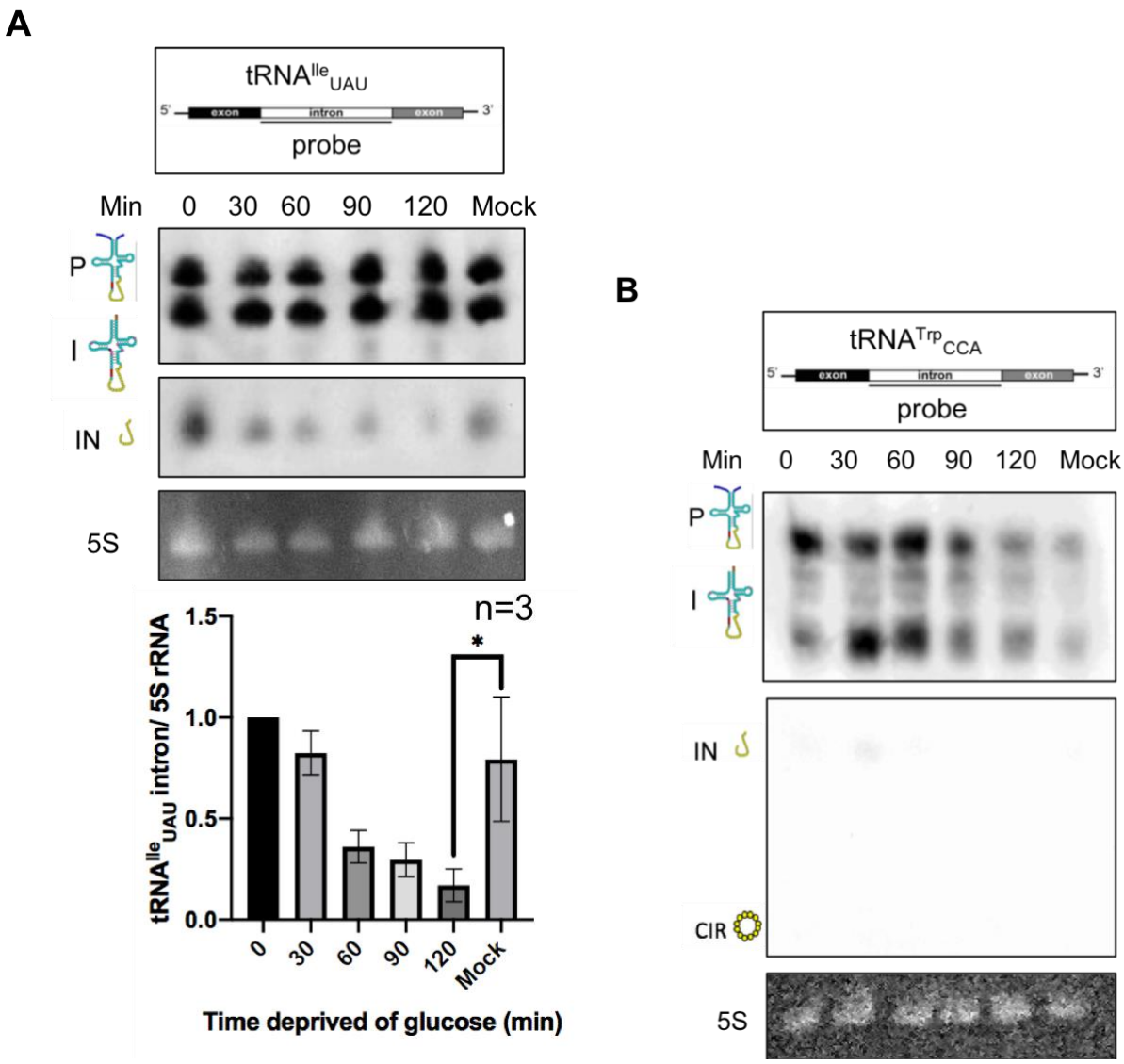



**Figure 8:** Rapamycin stress does not alter tRNA intron levels. **(A)** $tRNA_{IleUAU}$ precursor transcript levels decrease after 90 minutes of rapamycin stress. No significant change seen in $tRNA_{IleUAU}$ intron levels. **(B)** $tRNA_{TrpCCA}$ linear and circular intron levels not significantly affected by rapamycin stress. Precursor transcript levels show a decrease upon rapamycin stress. **(C)** $tRNA_{LysUUU}$ precursor transcript levels decrease in rapamycin-containing media. $tRNA_{LysUUU}$ free intron levels are unaffected. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.


A
tRNA$^{Ile}_{UAU}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
5S
n=3
*
tRNA$^{Ile}_{UAU}$ intron/ 5S rRNA
1.5
1.0
0.5
0.0
0 30 60 90 120 Mock
Time deprived of glucose (min)
B
tRNA$^{Trp}_{CCA}$
5'
exon
intron
exon
3'
probe
Min 0 30 60 90 120 Mock
P
I
IN
CIR
5S

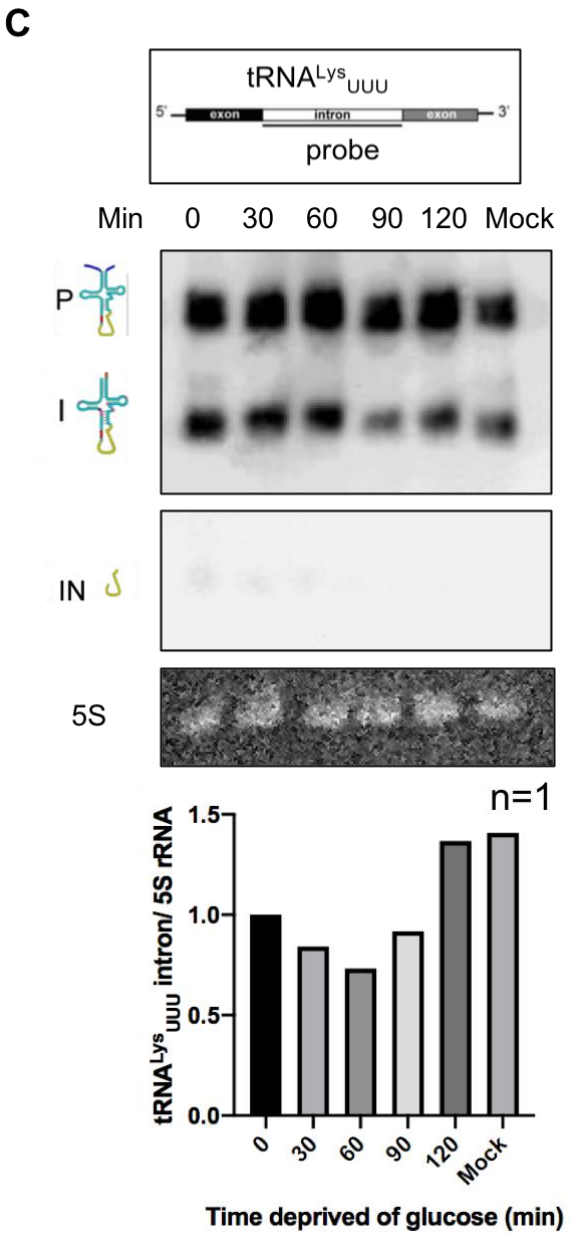



**Figure 9:** Glucose starvation causes more rapid turnover of tRNA$_{IleUAU}$ introns. **(A)** tRNA$_{IleUAU}$ intron levels gradually decrease upon starving cells for glucose. **(B)** tRNA$_{TrpCCA}$ linear and circular intron levels are unchanged by glucose starvation. **(C)** tRNA$_{LysUUU}$ intron levels are unaffected by glucose starvation. Quantification is normalized to the 0 minute ratio of tRNA intron level/ 5S rRNA level.

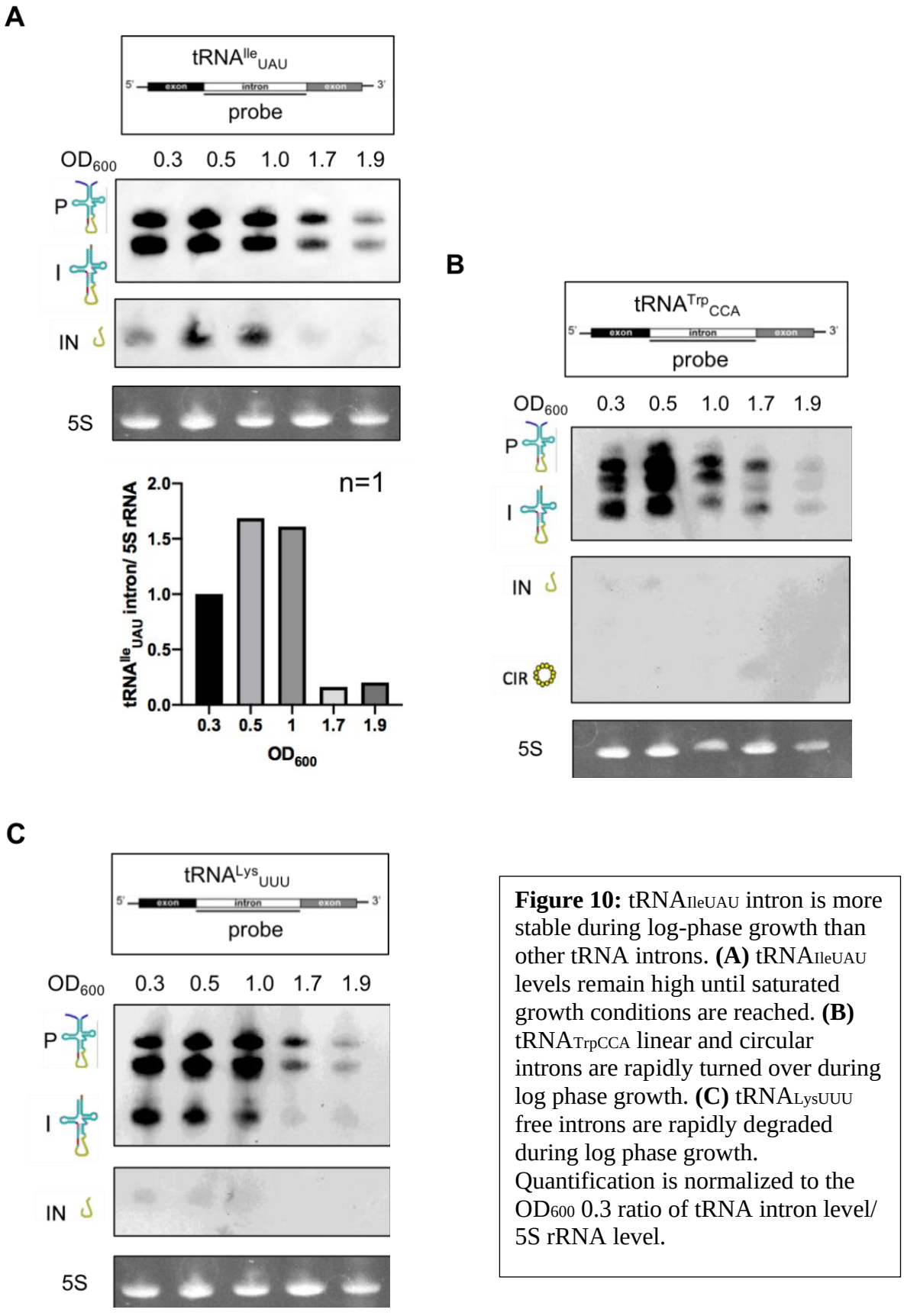



**Figure 10:** tRNA$_{IleUAU}$ intron is more stable during log-phase growth than other tRNA introns. **(A)** tRNA$_{IleUAU}$ levels remain high until saturated growth conditions are reached. **(B)** tRNA$_{TrpCCA}$ linear and circular introns are rapidly turned over during log phase growth. **(C)** tRNA$_{LysUUU}$ free introns are rapidly degraded during log phase growth. Quantification is normalized to the OD$_{600}$ 0.3 ratio of tRNA intron level/ 5S rRNA level.